Laboratory Techniques for:

1. Preparation of Agar
2. Sterilization of agar
3. Dilution plate method of spore transfer.
4. Transferring of fungus culture
5. Methods of sterilization of plant tissue for cultivation

I Dissolve 17g of agar in 500 cc of distilled water by carefully boiling or steaming. 400g of diced potatoes are boiled in 500 cc of H_2O and then strained. The potato decoction is added to the agar solution and the whole concoction is brought up to 1000 cc, filtered through a cloth and finally 20g of dextrose is added.

II Test tubes are filled ⅓ full of hot agar medium, plugged with cotton and placed in an autoclave and sterilized under pressure in steam. Upon removal from the autoclave tubes are placed at a near horizontal angle and left to harden. This produces more surface upon which the fungus etc. may grow. A hot water bath of 45° may be used to keep the agar in a moulten state if desired.

III

Make a suspension of spores by transferring a quantity of spores into a drop of water using a sterile needle. A sterile innoculating needle with a small loop on the end is then sterilized in a flame. Previous to this the table where transfers is to be made should have been sterilized with a mercuric bichloride. The cotton is pulled from the ends of two tubes (one containing melted agar medium and the other concentrated spore solution) and the mouths are sterilized over flame. The sterile needle is then used to transfer a small quantity of concentrate to the agar. It is mixed thoroughly with the agar by stirring with the needle. This is repeated again, a loopful of culture being transferred to a new agar solution. This is done several times and each time the new solution is emptied into a sterile petri dish and allowed to harden. When the cultures begin to show spore colonies, one colony may be transferred to a fresh tube of agar thus giving a pure culture. When making these transfers it should always be remembered that the mouths of all tubes should be sterilized both before and after transfer, the same operation being performed on the needle.

IV

Sterilize a needle and then dip it into agar to wet it.

Then (this is all done rapidly) touch a spore mass with the needle and scrape it several times across a tube of agar. Again it is essential to sterilize the mouth of the tube before and after transfer.

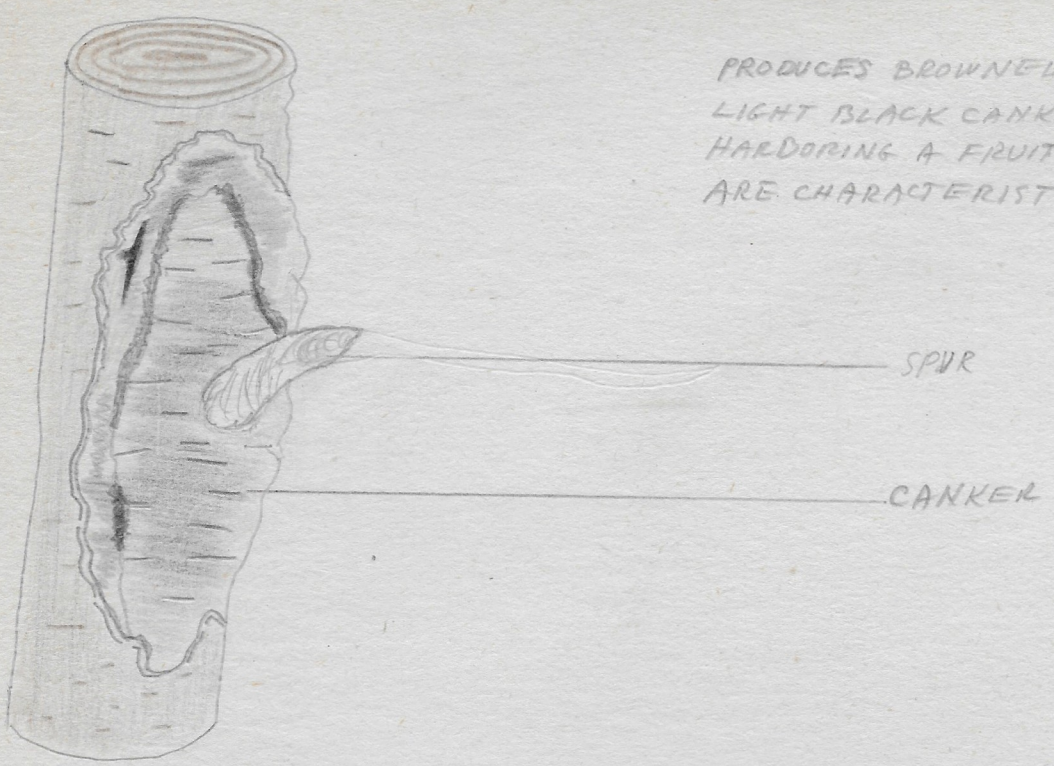
V

When a tissue transfer is to be made the tissue should first be sterilized to lessen the chance of transferring unwanted spores with the principal material. This surface sterilization may be accomplished by wiping the tissue with a cloth or cotton damp with alcohol. Another method of sterilizing tissue is to wash it back and forth through distilled water. Yet another method is to cut off a small portion of the material to be transferred and drop it for a minute or two into a calcium hypochlorite solution. This type of transfer is always made with sterilized instruments.

CANKER

FIRE BLIGHT OF APPLE
ERWINIA AMYLOVORA

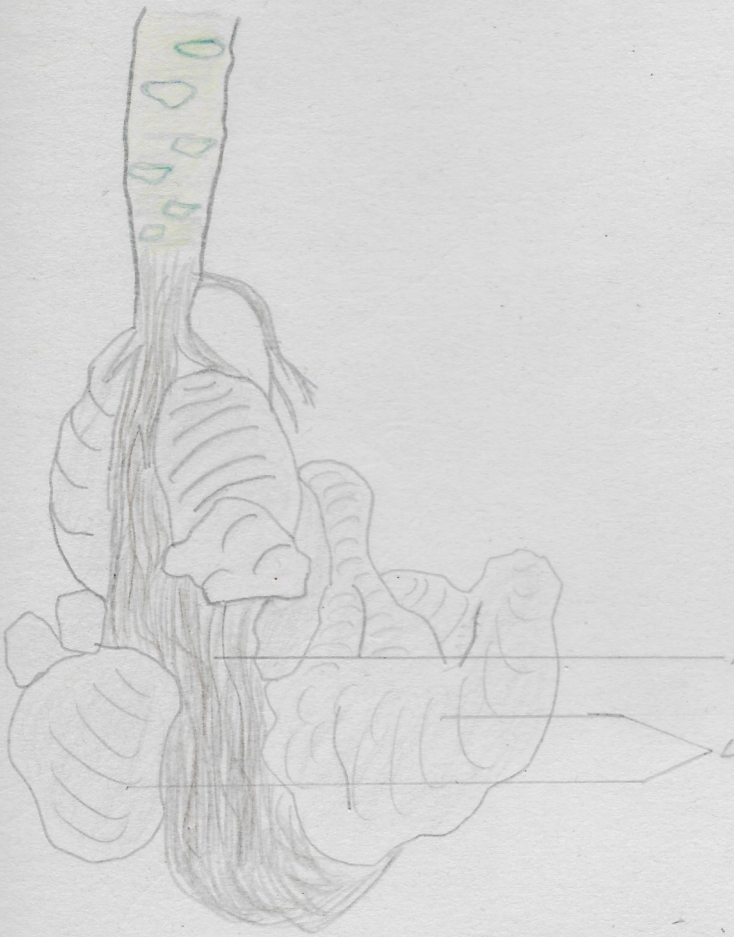
PRODUCES BROWNED DEAD LEAVES AND FR
LIGHT BLACK CANKERS WITH CRACKED BOR
HARBORING A FRUIT SPUR IN THE MIDDLE
ARE CHARACTERISTIC



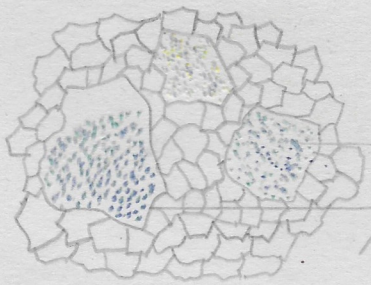
CANKER, DEAD + LIVE LEAVES + FRUIT

CLUB ROOT OF CABBAGE

PLASMODIOPHORA BRASSICAE

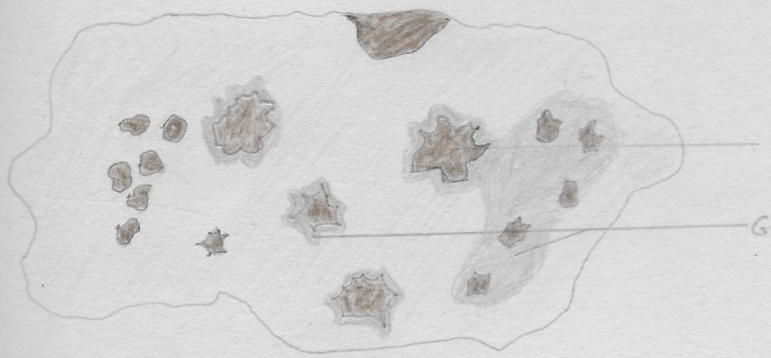


VEGETATIVE BODY OF CLUB ROOT



DISEASE ENTERS PLANT THROUGH ROOT HA
HOST CELLS ARE STIMULATED TO EXCESSIVE
DEVELOPMENT AND FOOD MATERIALS ARE
DRAINED FROM AERIAL PARTS OF THE
PLANT RESULTING IN LARGE BULBOUS
LIGHT BROWN OR DARK ROOT AREAS

POWDERY SCAB OF POTATO



CROWN GALL + HAIRY ROOT
AGROBACTERIUM TUMEFACIENS
AGROBACTERIUM RHIZOGENES

FECTED
TATO ROOT
STEM

CROWN GALL:
SWOLLEN ROUNDED ROUGHENED BULBOUS
SWELLING UP TO SEVERAL INCHES IN SIZE
A LITTLE DARKER THAN ORDINARY COLOR

HAIRY ROOT:
FINE ROOTS (HAIR-LIKE) WHICH REPLACE NORMA
ROOT SYSTEM MAY COME OFF ANY KNOT

SCAB OF POTATO

ACTINOMYCES SCABIES (THAX.) GRISSOW

INFECTED POTATO

SMALL BROWNISH SPOTS THAT ENLARGE + FORM HARD CIRCULAR OR IRREGULAR CORKY AREAS ON TUBER MAY CRACK OPEN

INFECTED AREAS

CROSS SECTION OF INFECTED POTATO

INFECTED AREAS

RED STELE OF STRAWBERRY
PHYTOPHTHORA FRAGARIAE (HICKMAN)

INFECTED PLANT

LEAVES ARE DWARFED AND WILTED (OLDER ONES) SLIGHTLY BLUISH IN COLOR. THE RED CORE IS DISTINCTIVE.

CROSS SECTION OF STEM

LATE BLIGHT OF POTATO
PHYTOPHTHORA INFESTANS

INFECTED POTATO

A BLACKENED OR BROWNED TUBER WHICH IS BROWNED AROUND THE INSIDE

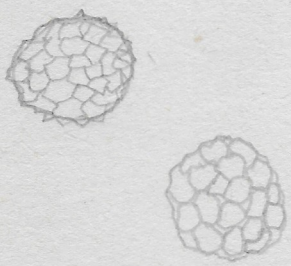
CROSS SECTION OF INFECTED POTATO

RHIZOPUS NIGRICANS

CONJUGATION & ZYGOTE

PERONOSPORA VICIAE

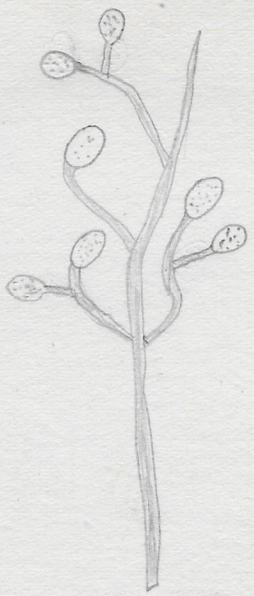
OOSPORES



DOWNY MILDEW OF GRAPE
PLASMOPORA VITICOLA

GRAPE INFESTATION

CONIDIOPHORE WITH CONIDIA



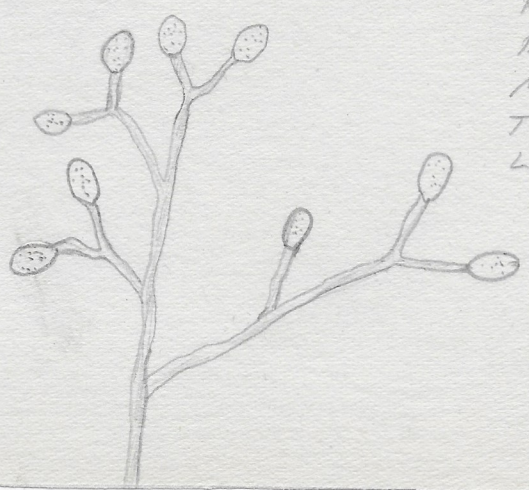
DOWNEY MILDEW OF CUCUMBER
PSEUDOPERONOSPORA CUBENSIS

UNDERSIDE OF LEAF

UPPERSIDE OF LEAF

RATHER INDISTINCT BROWN SMUDGES GRA
MASSES OF CUPS ON THE UNDERSIDE OF LEAVES

CONIDIOPHORE WITH CONIDIA



APPLE SCAB
VENTURIA INAEQUALIS

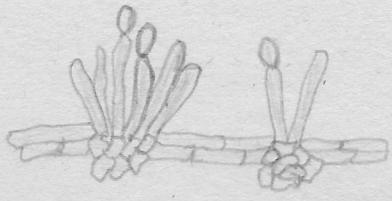
INFECTED LEAF

ON LEAF PURPLISH OR BROWNISH BORDER, RAISED CENTER AND GRAY RAISED CENTER (OLDER LEAVES HAVE THE DISTINCT SPOTS)

PERITHECIA AND ASCI

INFECTED APPLE

ACERVULVUS AND CONIDIA



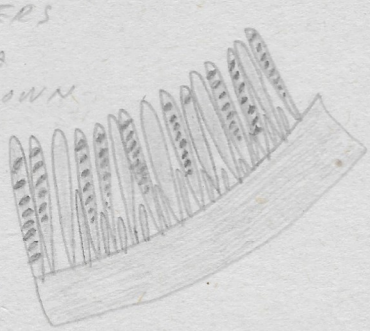
BROWN AND BLACK RAISED CORK-LIKE AREAS WITH A WHITE BORDER.

BROWN ROT OF PEACH
SCLEROTINIA FRUCTICOLA

BLIGHTED BLOSSOM AND CANKERS

REDDISH BROWN CANKERS FRUIT SPUR CANKERS A MORE CONSERVATIVE BROWN. BROWNED LEAVES AND BLOSSOMS

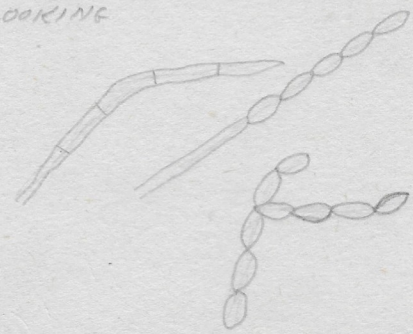
ASCI



INFECTED PEACH

BROWNED ROTTED AREA WITH LIGHT BROWN CONIDIA (LOOKING LIKE POWDER) ON IT

CONIDIA AND CONIDIOPHORES

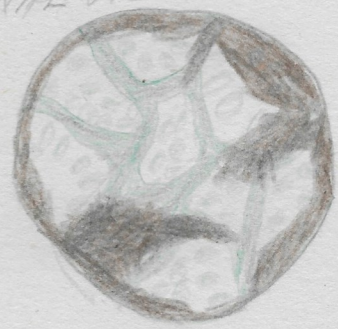


ASCUS + ASCOSPORES

LATE BLIGHT OF TOMATO
PHTOPHTHORA INFESTANS

INFECTED FRUIT
EXTERNAL VIEW

INFECTED FRUIT
INTERNAL VIEW



NO LEAF SPECIMEN AVAILABLE BUT
SYMPTOMS ARE SPOTS THAT RESEMBLE
OIL DROPPINGS ON LEAF.